

Fibrin Sealant Foam Sprayed Directly on Liver Injuries Decreases Blood Loss in Resuscitated Rats

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Objective: The majority of early trauma deaths are attributable to uncontrolled hemorrhage from truncal sites. A hemorrhage-control technique that reduced bleeding in the prehospital phase of treatment without requiring manual compression may improve the outcome of these patients. We conducted this preliminary study to determine whether an expanding fibrin sealant foam (FSF) would reduce bleeding from a severe liver injury even during resuscitation.

Methods: Rats ($n = 31$; 291 ± 5 g; $37.4 \pm 0.3^\circ\text{C}$; mean \pm SEM), underwent a $60 \pm 5\%$ excision of the median hepatic lobe. The animals received one of three treatments: (1) FSF, (2) immunoglobulin

G placebo foam (IgGF), or (3) no treatment. All animals were resuscitated with 40°C lactated Ringer's solution at 3.3 mL/min/kg to a mean arterial pressure of 100 mm Hg. Total blood loss, mean arterial pressure, and resuscitation volume were recorded for 30 minutes. A qualitative measure of foam coverage and adherence to the cut liver edge was recorded.

Results: The total blood loss was less ($p < 0.01$) in the FSF group (21.2 ± 5.0 mL/kg) than in either IgGF (41.4 ± 4.3 mL/kg) or the no treatment group (44.6 ± 4.7 mL/kg), which did not differ. The resuscitation volume was not different. The amount of foam used in the treated groups, 9.1 ± 1.0 g in the FSF group and

10.0 ± 1.0 g in the IgGF group, did not differ. Survival for 30 minutes was not different among groups. There was no difference in the amount of cut liver covered by either foam, but the clots were more adherent ($p < 0.05$) in the FSF group than in the IgGF group.

Conclusion: In rats with a severe liver injury, spraying fibrin foam directly on the cut liver surface decreased blood loss when compared with placebo foam and no treatment. This pilot study suggests a future possible treatment for non-compressible truncal hemorrhage.

Key Words: Hemorrhage, Injury, Liver, Hemorrhage Control, Hemostasis, Fibrin sealant foam, Animals, Rats

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Hemorrhage remains the greatest threat to life in the first 24 hours after severe injury.¹ Many of these deaths occur before patients reach the hospital.^{2,3} Current attempts to decrease the frequency of these early deaths have focused on optimal resuscitation and/or rapid transport. Although urban scene and transport times combined are now

often under 20 minutes, patients still arrive in hospitals hypotensive, with up to a 50% mortality rate.^{3,4} Unfortunately, the methods currently available to prehospital personnel for controlling hemorrhage (tourniquets, pressure points, and pressure dressings) have not changed for many years.^{5,6} These methods are only useful for extremity or superficial truncal injuries, which account for only 10% of combat hemorrhagic deaths and even fewer in civilians.^{7,8} Up to 90% of all hemorrhagic deaths in Vietnam were from truncal injury.⁷ First responders (emergency medical technicians or combat medics) have no means at their disposal to stop truncal hemorrhage. While treating hypotensive bleeding patients, field personnel can only control the airway and resuscitate and then transport the patient to a hospital.⁹ Therefore, patients with serious truncal wounds often arrive in operating rooms significantly hypotensive from persistent blood loss.

Recently, there has been increasing interest in improving the hemorrhage control techniques available to surgeons in the operating room. Liquid fibrin sealants have been used effectively to control splenic and hepatic bleeding in trauma patients.^{10,11} However, because liquid fibrin sealants coagulate immediately after leaving the delivery syringe, this method relies on direct injection into, near, or on the visualized bleeding surface and, therefore, is applicable only in the operating room. Because of the low tensile strength, handling difficulties, and logistic problems of liquid fibrin sealants, the authors developed a dry fibrin sealant dressing and have investigated its effectiveness for reducing blood loss from

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large and rapidly bleeding extremity, kidney, prostate, and liver injury models.^{12–16} However, the dry fibrin sealant dressing is only applicable where pressure dressings can be manually applied to accessible and compressible hemorrhaging sites.¹⁷

Because no method currently exists to stop truncal or noncompressible hemorrhage in the field or emergency department, we developed a self-expanding fibrin sealant foam (FSF) whose composition was based on the dry fibrin sealant dressing concept. This material could potentially be introduced into a body cavity by a trocar, spread throughout the cavity, and stop the bleeding from sites inaccessible to direct pressure. As a first step in demonstrating the potential utility of this approach, it was necessary to establish that a FSF could stop bleeding from a solid organ. We hypothesized that the FSF would control bleeding in the absence of direct pressure by bonding to damaged tissue, thus decreasing blood loss when compared with a control foam and no treatment. This preliminary study was performed to evaluate the effectiveness of FSF when sprayed directly on a severe liver injury in rats.

MATERIALS AND METHODS

Thirty-six Sprague Dawley rats (260–310 g) were used in this study. Animals were housed inside in a climate-controlled facility. Food and water was available ad libitum. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility. The protocol was approved by the Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Ft. Sam Houston, TX. All animals received care in strict compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Animals were assigned to treatments in the study according to a random number table. Treatment groups were designated as the following: (1) fibrin sealant foam group (FSF), (2) immunoglobulin G foam group (IgGF), and (3) no treatment control group (NT). The IgGF was a placebo foam and differed from the FSF in that an equal mass of IgG was substituted for fibrinogen. Thus, the two foam preparations had the same total quantity of plasma protein, including thrombin, and were visually identical to each other before and during application. The IgG has no known hemostatic qualities, even when mixed with thrombin. The investigators were blinded to treatment for groups 1 and 2.

Anesthesia was induced with pentobarbital sodium (60 mg/kg intraperitoneally) administered through a 25-gauge needle. Carotid arterial and jugular venous catheters were placed via cervical cutdown. The internal jugular line (25 gauge) was connected to a pump for resuscitation. A rectal temperature probe was placed and connected to a digital thermometer. The preinjury temperature was maintained at 37.5°C with a warming blanket and heat lamp. Mean arterial pressure (MAP) and systolic and diastolic blood pressures as well as heart rate were recorded at 10-second intervals throughout the study period using a continuous data collection system (Micro-Med, Louisville, KY) connected to the

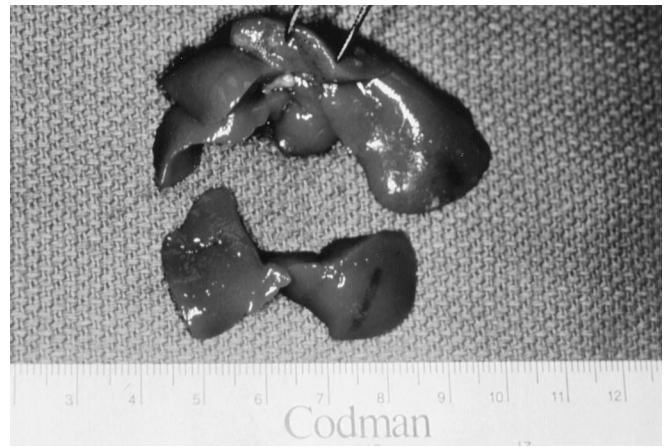


Fig. 1. Postmortem rat liver specimen, with the forceps pointing to the cut surface of the median lobe. Separate lower section represents the excised portion of liver.

arterial line. The liver injury model developed by Matsouka et al. was modified, yielding a simple, reproducible, nonheparinized severe liver injury model of uncontrolled hemorrhage.¹⁸ After adequate anesthesia was induced, a mid-line laparotomy was performed. Utilizing a small plastic ruler, the capsule of the median lobe was scored in three spots (lateral, medial, and in the midline), 1 cm from the suprahepatic vena cava, with a handheld cautery. The abdominal cavity was wiped dry with a 2 × 2 gauze sponge, and the portion of the median lobe distal to the previously placed marks was sharply excised with scissors. No manipulation of the rapidly bleeding liver occurred. Removal of this portion of the median lobe resulted in an easily visualized, ventrally located crescent-shaped cut portion of liver, approximately 30 × 5 mm (Fig. 1).

As one investigator created the hepatic injury, the components of the foam were mixed and then sprayed directly on the cut surface of the liver immediately after the liver was excised. No blood was removed from the peritoneal cavity or cut liver surface before foam application. The liver was actively bleeding as the foam was applied. The abdominal cavity was left open, and single gauze 2 × 2's were used as necessary to collect blood if it overflowed the peritoneal cavity. Resuscitation in all animals with warm (40°C) lactated Ringers was started (3.3 mL/min/kg) via the internal jugular line immediately after completion of the liver injury. The end point of resuscitation was a MAP of 100 mm Hg. This resuscitation regimen was continued until the goal was reached, and reinitiated if MAP decreased during the 30-minute study period. After the liver injury, the animals were monitored for 30 minutes or until death, whichever came first. Death before 30 minutes was defined as a respiratory rate of 0 and no pulse. After 30 minutes, the animals that were still alive were euthanized with an overdose of sodium pentobarbital (Sleepaway, 0.5 mL intravenously; Ft. Dodge Laboratories, Riverside, IA).

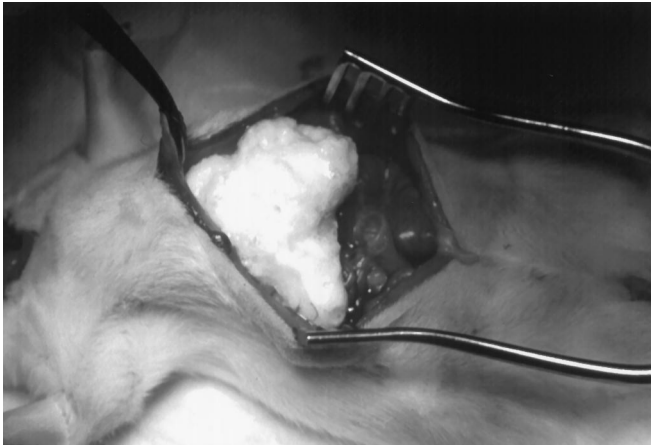


Fig. 2. Fibrin sealant foam (FSF) filling the peritoneal cavity after liver injury.

The foam was produced by simple, rapid mixing of the dry components (total weight, 2.82 g) with saline (30 mL of 0.9% normal saline). The dry components of the FSF consisted of human fibrinogen, human thrombin, CaCl_2 , and the gas-generating chemicals and were stored dry at room temperature. When mixed with saline, the hydrated ingredients generate a foam comprising 1.7 mg/mL CaCl_2 , 1.9 mg/mL of fibrinogen, and 36 IU/mL of thrombin. The saline was added to the dry ingredients in a canister with a short nozzle. This mixture immediately generated a fibrin foam that was self-propelled through the nozzle and onto the bleeding liver surface. The resulting foam squirted under its own pressure through a nozzle held 5 cm from the cut surface of the liver (Fig. 2). Over the ensuing 30 minutes, the gas bubbles dissipated and left only a very small residual material attached to the injured liver (Fig. 3).

At the end of the study period, shed blood in the abdominal cavity was removed with 2×2 sponges. The total blood loss was calculated as the difference between blood-soaked sponges minus the weight of preweighed dry 2×2 's for each

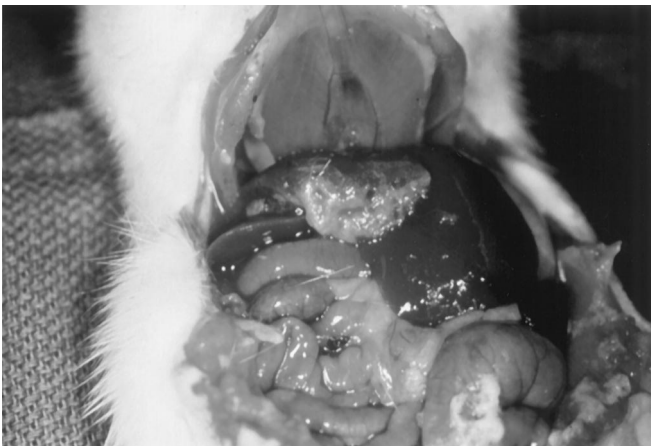


Fig. 3. Residual fibrinogen clot on cut surface of the liver 30 minutes after injury.

animal. Total resuscitation fluid used and time to death was recorded. The amount (grams) of foam applied was recorded as the difference in weight of the foam components and container immediately before and just after application. Each liver was removed and the remaining median lobe was dissected from the liver and each section individually weighed.

Two semiquantitative observations of the foam were performed by a single operator. A foam coverage score was recorded for each animal, expressed as a range of percent of surface covered, with the foam described as covering $<10\%$ of the cut liver surface = (1), 50% covered = (2), or $>90\%$ covered = (3). Foam adhesiveness was scored as loose = 1 or tight = 2. We also noted whether foam was adherent to noninjured peritoneal or bowel surfaces.

The weight of the excised median lobe divided by the preinjury total body weight of the rat was used as a measure of the reproducibility of the injury. Experience during model development revealed that results $<0.8\%$ or $>1.2\%$ yielded an injury that either resulted in insignificant bleeding or an animal that rapidly bled to death, respectively. Therefore, if the amount of excised liver was not within these criteria, the animal was excluded from further analysis. By calculating this ratio immediately after injury, animals falling outside the established parameters were prospectively excluded.

Blood loss was corrected for body weight (mL/kg). All measures are presented as mean \pm SEM. Differences in group means were determined with analysis of variance (SyStat Ver 6.0, SPSS, Inc, Chicago, IL). For measures with differences between group means, direct comparisons of the FSF group with other groups were performed using the Fisher least significant difference measure. Differences in the liver coverage and adherence of clot score were evaluated with the Wilcoxon rank sum test. Probabilities ≤ 0.05 are considered statistically significant.

RESULTS

Thirty-six rats were used in this study and 31 had the appropriate injury created. These included 9 FSF, 12 IgGF, and 10 NT animals. All further comments refer to these 31 animals.

Body weight, core temperature, and starting MAP were not different between treatment groups. The fraction of liver excised did not differ between treatment groups when measured as a fraction of median lobe weight, liver weight, or body weight. With injury, the mean lowest MAP or the mean time to reach the lowest MAP did not differ between treatment groups. This data is presented in Table 1.

Treatment results are presented in Table 2. The FSF reduced the blood loss by almost 50% compared with the control group ($p < 0.01$). The volume of resuscitation fluid administered was not different between the two groups. The foam coverage of the injury was not different between the active and inactive material, but the FSF adhered more firmly in a larger fraction of the animals ($p < 0.05$).

Table 1 Comparability of Groups Treated

	Treatment Group			<i>p</i> Value
	FSF	IgGF	NT	
n	9	12	10	
Weight (g)	291 ± 14	292 ± 12	290 ± 14	NS
MAP, starting (mm Hg)	132 ± 15	131 ± 22	140 ± 19	NS
MAP, lowest (mm Hg)	34 ± 15	24 ± 8	37 ± 14	NS
ML % excised	25.9 ± 0.8	26.6 ± 0.7	24.6 ± 1.0	NS
BW % excised	1.02 ± 0.03	1.00 ± 0.03	0.97 ± 0.03	NS

FSF, fibrin sealant foam; IgGF, placebo foam; NT, no treatment; MAP, mean arterial pressure; ML, median lobe; BW, body weight; NS, not significant.

DISCUSSION

This was a pilot study to determine whether a FSF could reduce bleeding from a solid-organ injury. In this study, the extent of liver injury and its physiologic consequences appear equivalent across all groups. The injury was designed to be an easily accessible, ventrally placed, broadly cut surface of exposed liver, allowing the sprayed foam easy access to the bleeding surface. Furthermore, there was no difference in the amount of foam sprayed onto the cut liver surface in either foam group. These quantitative assurances of comparable injury and treatment allow the differences observed in blood loss to be attributed to the effectiveness of the hemostatic method used. The principal outcome measure in this pilot study, the decrease in blood loss with use of the FSF compared with the use of IgGF or NT, was significant ($p < 0.01$).

To our knowledge, this is the first study to demonstrate that a hemostatic foam, without any form of manual compression, can decrease blood loss when applied directly onto the cut surface of an actively bleeding solid organ. This result may seem obvious, inasmuch as many others have shown that fibrin sealant sprayed onto bleeding surfaces reduces blood loss.^{19–31} In fact, this is why the Food and Drug Administration recently licensed one of the liquid products. However, the liquid fibrin sealant mixtures must be applied directly on or injected near an injury, as cross-linking starts immediately. Ultimately, we anticipate that the FSF will be sprayed into a body cavity and spread throughout, bonding to only damaged surfaces and forming a hemostatic plug. In this preliminary study, the FSF appears to meet the first requirement of effective hemostasis. The coverage and adherence scores reveal

that the fibrinogen and IgG foam covered the surface of the liver equally; however, the FSF stuck tightly to surface of the damaged liver, whereas the IgG foam did not.

Despite the differences in blood loss between the groups, there was no difference in the number of animals that died during the study period or resuscitation fluid use within groups. This was probably because of the short time period of the pilot study. In fact, given the volumes of blood lost and the rates of fluid resuscitation, the typical 3:1 replacement of blood loss with crystalloid solutions could not have been complete in the IgGF and NT groups in the 30 minutes of the study.

The actual process by which a FSF stops bleeding has not been studied, but probably involves the bonds formed between cross-linked fibrin and damaged collagen seen with standard fibrin sealant preparations.^{19,20} The cross-linking may explain why the FSF only stuck to injured liver and not to other nondamaged intraperitoneal surfaces. We think this is the basis for the hemorrhage control provided by the FSF. Importantly, although the FSF stuck tightly to the damaged liver surface, it did not stick to uninjured bowel or peritoneum; within 30 minutes of application, the foam had shrunk to a small fibrin clot only attached to the cut surface of the liver (Fig. 3). This work suggests that it may be possible to inject FSF into the closed peritoneal cavity as a prehospital or delayed-treatment hemorrhage control modality.

Previous reports of hypotensive reactions after the application of fibrin sealant to open wounds probably do not apply to this material and application.³² The authors have previously reported that this complication is related to kallikrein contamination of the bovine thrombin used in liquid fibrin sealants and

Table 2 Comparability of Treatment Results

	Treatment Group			<i>p</i> Value
	FSF	IgGF	NT	
Blood loss (mL/kg)	21.2 ± 5.0	41.4 ± 4.3	44.6 ± 4.7	<.01
Resuscitation volume (mL)	17 ± 8	19 ± 8	20 ± 6	NS
Foam volume (mL)	9.1 ± 1.0	10.0 ± 1.0		NS
Wound foam coverage	50–90%	50–90%		NS
Wound clot adherence	Tight	Loose		<.05

FSF, fibrin sealant foam; IgGF, placebo foam; NT, no treatment; MAP; NS, not significant.

the manufacturer of the implicated product has removed it from the market after confirming the findings (unpublished data).^{33,34}

This small pilot study of a novel form of hemorrhage control raises many more questions than it answers. The most important and most difficult to answer is will this hemostatic foam technique be effective when applied into a closed chest or abdomen? Equally important will be identifying groups of patients that may benefit from this approach. Questions about long-term safety include whether an increase in abdominal compartment pressures is produced and whether foam in the abdomen prevents the subsequent operating surgeon from identifying injured structures. Delivery devices and optimal methods and tools to guide application all warrant further study. Furthermore, the combination or tradeoffs of FSF combined with hypotensive resuscitation techniques may deserve evaluation.

Despite these and other questions, it remains clear that uncontrolled truncal hemorrhage is a major cause of death on the battlefield and in civilian trauma patients. The current hemorrhage control techniques available outside the operating room are of no benefit for the majority of soldiers and civilians dying of noncompressible truncal hemorrhage. If we hope to decrease the mortality of severely injured patients, one of our objectives must be development of simple, rapid and field expedient techniques for nonsurgeons to stop truncal hemorrhage. This preliminary study is a first step down that pathway.

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